Life History and Cost Analysis for Continuous Rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a Zoophytophagous Artificial Diet

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ABSTRACT The impact of a zoophytophagous, insect-free artificial diet upon the developmental rate, life table parameters, and fertility table parameters was examined over 11 consecutive generations for domesticated and wild colonies of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). This study showed that the developmental time, preoviposition period, fecundity, and nymphal survival improved in the domestic colony when fed an insect-free artificial diet for 11 consecutive generations, but remained relatively constant for the wild colony, as did reproductive rate and intrinsic rate of increase. Although, after 11 generations of adaptation to an artificial diet feeding regime, all reproductive and fertility table parameters were still significantly lower than when fed on *T. ni* larvae as the natural prey, the realized cost of rearing either colony on the artificial diet approached 1.2 times the cost of rearing these insects on a natural prey. This is a significant achievement in the effort to develop cost-effective artificial diets for the mass-rearing of beneficial pentatomids, and has positive implications for the use of one artificial diet to efficiently rear several beneficial insects.

KEY WORDS beneficial insect, predator, intrinsic rate of increase, fecundity, cost efficiency

LARGE NUMBERS OF BENEFICIAL insects must be available at low costs for inoculative and augmentative releases of insects to become viable as an alternative to chemical pesticide usage. The cost of rearing beneficial insects on natural prey is often too high to compete with the cost of chemical control. However, rearing cost could be reduced substantially with inexpensive, efficient diets to replace the cost of rearing natural prey as food sources (Glenister 1998, Glenister and Hoffmann 1998, Ruberson and Coll 1998, Thompson 1999).

Various artificial diets have been developed for the maintenance and propagation of predators and parasitoids. Although there has been some success in efforts to rear successive generations of some parasites and predators entirely on artificial diets, in many cases there is a significant loss of both fitness and reproductive potential; that is, longer developmental time and lower fecundity (De Clercq and Degheele 1992; Carpenter and Greany 1998; Thompson 1999; Adams 2000a, 2000b; Rojas et al. 2000), which reduces the cost savings associated with the use of an artificial diet. Life tables and fertility tables have been shown to be powerful tools for analyzing and understanding the impact that an external factor, such as an artificial diet, has upon the growth, survival, reproduction, and rate of increase of an insect population (Landahl and Root 1969, Bellows et al. 1992). Life table and fertility tables have been used to improve rearing techniques (Birch 1948) and compare different food sources (Fouly et al. 1995, Valicente and O'Neil 1995, Hodek and Honek 1996, Souissi and Le Ru 1997, Richard and Evans 1998, Hansen et al. 1999).

Podisus maculiventris is considered a good candidate for augmentative release in the control of Colorado potato beetle and many other agricultural pests (Mukerji and LeRoux 1969; Waddill and Shepard 1975; McPherson 1980, 1982; Drummond et al. 1984; De Clercq and Degheele 1992, 1997; Hough-Goldstein and McPherson 1996; De Clercq et al. 1998b; Yeargan 1998) because of its high reproductive capacity and its ability to be reared on artificial diets (Hough-Goldstein and McPherson 1996, Hough-Goldstein 1998, De Clercq et al. 1998a). *P. maculiventris* is referred to as an entomophagous predator (i.e., carnivorous feeder), yet this species is often observed probing (puncturing with the stylet) the plants that provide nutrients for the host insect (Ruberson et al. 1986). It is commonly accepted that plant probing by this entomophage is to acquire water. However, from a nutritional perspective it remains unresolved whether the feeding habits of this insect are solely entomophagous,

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or carnivorous and phytophagous (i.e., zoophytophagous) (Miles 1972, Cobben 1978).

Thus far, artificial diets developed for the continuous rearing of *P. maculiventris* have been devoid of insect and plant material, but rather are composed of meat and egg ingredients, and have adversely affected nymphal development and reproductive capacity (De Clercq and Degheele 1992, De Clercq et al. 1998a, Wittmeyer et al. 2001). Additionally, a prior study of the performance of *P. maculiventris* on an artificial diet comprised of a blended buffered mixture of beef liver and whole egg showed that the "realized" cost to rear *P. maculiventris* on the artificial diet (calculated as the cost to double the population size) was 3.5 times higher than the cost of rearing on natural prey because of a decrease in the intrinsic rate of increase brought on by the prolonged developmental time and reduced reproductive output (Wittmeyer and Coudron 2001).

Because *P. maculiventris* frequently probes plants, it is possible that this entomophagous insect retrieves some substance(s) from the plant, as well as nutritive material from the insect host. After numerous field observations of *P. maculiventris* probing plants, we decided to test a zoophytophagous artificial diet, devoid of insect material, but comprised of plant material, bovine liver, and egg as the nutritive sources. In this study fertility table parameters were used to examine the impact of the zoophytophagous diet upon the developmental rate and rate of increase of *P. maculiventris.* In addition, the intrinsic rate of increase, converted to doubling time, was used to calculate the realized cost of rearing (calculated as the cost to double the population size), to evaluate the cost efficacy of the artificial diet tested.

Materials and Methods

Insect Colonies and Diet. Experimental specimens were obtained from a domesticated colony and from field collections of *P. maculiventris.* The lab colony (domesticated specimens) was taken from a colony maintained in culture for >350 generations. The wild colony (field specimens) was initiated with eggs oviposited by females collected in an alfalfa field near Columbia, MO. Colony and experimental rearing conditions were 26 ± 5°C, 65 ± 10% RH, and a photoperiod of 16:8 (L:D) h, maintained in a walk-in growth chamber.

The zoophytophagous diet used in this study was a blended buffered mixture of a plant-based meridic diet (Debolt 1982) to which meat (beef liver) and whole egg (Wittmeyer and Coudron 2001) were added. The diet was encapsulated in a Mylar- (Clear Lam. 1992, Jefferson Smurfit Corp., Schaumburg, IL) Parafilm (52858-032, American National Can., Chicago, IL) dome of 40 μl volume as previously described (Wittmeyer et al. 2001, Wittmeyer and Coudron 2001). The natural prey used were fourth instar larvae of *T. ni* coddled at 60°C for 60 s to kill larvae and prevent feeding of isolated nymphs by live *T. ni* larvae. Isolated individual prey-fed nymphs and adults were given two coddled larvae per predator every 24 h. Artificial diet domes were replaced every 48 h; at each feeding one dome was given to the second and third instars, two domes were given to the fourth instars and four domes were given to the adult insects.

Experimental Design. Eggs were collected from the colonies, hatched, and first instar nymphs (previously demonstrated to require only water for survivorship to second instar) were placed in half-pint paper containers containing a moist dental wick. Randomly selected samples of 100 newly ecdysed second-instar nymphs from each colony (i.e., lab and wild), collected within 8 to 12 h of molting, were isolated in half pint paper containers and used to begin the treatments (i.e., for both prey [i.e., *Trichoplusia ni*] and diet [insect-free artificial media] treatments), as previously described (Wittmeyer et al. 2001). Preliminary data from previous observations indicated that the life history parameters of lab and wild colonies reared on natural prey remained stabled over successive generations. Therefore, only F1 data were collected for the prey-fed colonies. For the F1 generation of prey-fed and diet-fed lab and wild insects, and the F11 generation of diet-fed lab insects, a random sample of 30 nymphs per treatment was weighed individually on the day of eclosion to the second instar and 5, 10, and 15 d thereafter. By day 20 nymphs from each colony had become adults. If an individual selected for weighing died during the experimental time, no weights of replacement insects were taken. Daily observations of development and mortality were made for all individuals. Molting was recorded daily, as determined by the presence of exuviae. Developmental time of nymphal stages was measured as time (days) within each stadium, and time (days) from eclosion o f the second instar to adult eclosion. Life table values of l_x (number of individuals alive at beginning of stage x) and d_x (number of individuals dying in stage x) were obtained for each stage (x), and used to calculate stage specific mortality and generational mortality of nymphs.

For the F1 generation of prey-fed and diet-fed lab and wild insects, and the F six and F11 generations of diet-fed lab and wild insects, individuals were sexed at adult emergence and weighed 3 d later. Five days after adult molt, 20 females were paired with males of the same treatment and allowed to mate with the same male for 8 h within each successive 48 h during a 12 d period, for a total of six mating trials. Males were held individually and fed separately when not mating. Dead males were replaced with virgin males of similar age and treatment. Mortality of females was recorded daily and dead females were not replaced. Eggs were collected daily for 12 d after the initiation of mating (up to 17 d postemergence of adult females). Eggs were counted, observed daily for hatch, and first instar nymphs were observed until eclosion to the second instar.

To examine the impact of the artificial diet upon population growth, fertility table parameters were calculated for each treatment. All life table and fertility table parameters were measured and calculated as

Table 1. Effect of insect colony and food source on nymphal weight[a]

Main effect	day 0 (df = 1,148)	day 5 (df = 1,143)	day 10 (df = 1,139)	day 15 (df = 1,136)
Colony (wild vs. lab)	$F = 6.19$ ($P = 0.01$)	$F = 25.43$ ($P < 0.0001$)	$F = 10.12$ ($P = 0.001$)	$F = 54.77$ ($P < 0.0001$)
Food source (prey vs. diet)	$F = 10.06$ ($P = 0.001$)	$F = 549.82$ ($P < 0.0001$)	$F = 226.90$ ($P < 0.0001$)	$F = 98.38$ ($P < 0.0001$)

[a] GLM analysis.

described in Birch (1948) and Abou-Setta et al. (1986).

Statistical Analysis. All statistics were performed on SAS system software (1989–1996 by SAS institute Inc., Cary, NC). Original data were tested for normality using an univariate analysis and the W-test for normality (Shapiro and Wilks 1965, SAS 1982), and rank transformation (Spearman's rank correlation analysis) was applied to data that displayed non-normality and skewed distribution frequency (Conover and Iman 1981). Rank transformed data for nymphal weight and developmental time (for males and females separately, and males and females combined) were evaluated using a general linear model (GLM) to test the effects of treatment (i.e., treatment prey-fed and diet-fed as the independent variable) with a significance level at $P = 0.05$ (Wittmeyer and Coudron 2001). Stage-specific mortality and generation mortality were analyzed using a Log-Rank Test (SAS Institute 1995). For rank-transformed data (i.e., developmental time of females that were mated, preovipositional adult weight, preoviposition period, and eggs per female) a GLM was used on transformed data to test the effect of treatments. Fertility table age specific fecundity, m_x values (the number of female eggs laid per female at time x), include all fertile and infertile females to provide a more accurate estimate of reproductive rate (R_0), mean generation time (T) and intrinsic rate of increase (r). The number of females evaluated for reproductive parameters per treatment was 20 per generation.

Analysis of Rearing Cost. The estimated cost of production was calculated assuming minimum colony sizes for prey (six cages of adults with 100 females per cage and cages replenished weekly) and test insects (10 cages of adults with 12 females per cage and replenished weekly), and by not including overhead facility costs and salaries of workers (Wittmeyer and Coudron 2001). The estimated cost to maintain a *T. ni* colony was determined to be \$3.21/d and the estimated cost to produce the artificial diet was determined to be \$3.68/d. It was also estimated to cost \$2.57/d to feed *P. maculiventris* colony with either source of food, *T. ni* larvae or artificial diet.

The total cost per generation (TCG) for all treatments was calculated by the following equation (Wittmeyer and Coudron 2001). $TCG = (n_e)(c_t) + (n_n)(c_f+c_t+c_d) + (n_a)(c_f+c_t+c_d)$; where n_e = number of days as eggs and first instar (7 d), c_t = cost per day to maintain *T. ni* colony (if prey-fed, then c_t = \$3.21; if diet-fed, then c_t = \$0), n_n = number of days as nymphs from molt of second instar to adult molt, n_a = number of days as adults (17 d for both prey-fed and diet-fed), c_f = cost to feed *P. maculiventris* when fed either food source (\$2.57), c_d = cost per day to produce artificial diet (if prey-fed, then c_d = \$0; if diet-fed, then c_d = cost of diet production).

For all treatments, cost per egg (CPE) was then calculated by the after equation: $CPE = (TCG)/[(e)(f)(s)]$; where e = average number of eggs laid per fertile female; f = number of fertile females surviving to end of egg laying period, and s = proportion of eggs laid that survived the molt to the second stadium.

To evaluate the effect of the intrinsic rate of increase upon the cost of colony maintenance, the doubling time, (T_d, the number of generations required for the population to double in size) was calculated for prey-fed and diet-fed insects using above-derived r values and T values inserted in the equation $T_d = [(\ln 2)/r]\ (1/T)$ (Wittmeyer and Coudron 2001). The "realized" cost (the cost to double the population size) was determined as the total production cost per generation multiplied by the doubling time (T_d) in units of generation. The plots of R_o, T, r, T_d, cost to double, and cost per egg were fitted to a trendline most accurately following the pattern of the plot, either linear or exponential, and an estimated generation for when diet-fed values will reach prey-fed values was calculated from the predicted trendline equations.

Results

Effect of Artificial Diet on Nymphal Weight. The treatment effects over the 15 d observation period for nymphal weights were the same for both males and females analysis of variance (ANOVA); $F = 1.08$, df = 1, 543, $P = 0.3004$). Therefore, the data for male and female weights were pooled for these analyses. Both insect colony and food affected the nymphal weight (Table 1). The nymphal weight of wild insects exceeded that of lab insects at 0, 5, 10, and 15 d postemergence. The greatest difference was observed at day 15, with a mean difference of 12.751 between the two groups. The nymphal weight of prey-fed insects exceeded that of diet-fed insects at 0, 5, 10, and 15 d postemergence. The greatest difference was observed at day 15, with a mean difference of 15.19 between the two groups.

Significant treatment effects (Fig. 1) were found for the F1 mean nymphal weights between prey-fed and diet-fed at 0, 5, 10, and 15 d postemergence for lab insects (F-test; $F = 11.35$, df = 1, 58, $P = 0.0013$; $F = 408.04$, df = 1, 53, $P < 0.0001$; $F = 310.11$, df = 1, 49,

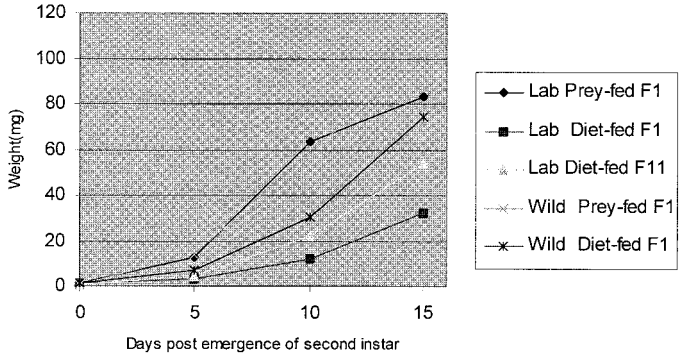

Fig. 1. Nymphal weight of F1 and F11 lab and F1 wild *P. maculiventris* reared on larval prey or a zoophytophagous diet. Data points represent means of each treatment.

$P < 0.0001$; $F = 203.34$, df = 1, 46, $P < 0.0001$; respectively) and wild insects ($F = 16.64$, df = 1, 58, $P = 0.002$; $F = 193.48$, df = 1, 58, $P < 0.0001$; $F = 68.72$, df = 1, 58, $P < 0.001$; $F = 25.90$, df = 1, 58, $P < 0.0001$; respectively). The mean nymphal weights for diet-fed insects were higher in the lab and wild colonies only at the day of emergence. Significant differences were also found between the mean nymphal weights of F1 and F11 diet-fed lab insects at 0, 10, and 15 d postemergence ($F = 31.13$, df = 1, 51, $P < 0.0001$; $F = 21.43$, df = 1, 47, $P < 0.0001$; $F = 25.80$, df = 1, 52, $P < 0.0001$, respectively); where F11 had the highest weights at 10 and 15 d postemergence. No significant difference was found at 5 d postemergence ($F = 3.68$, df = 1, 51, $P = 0.0605$).

The equations of fitted lines for a linear regression of Log_{10} transformed nymphal weight data with x = 0, 5, 10 and 15 (days) were: F1 prey-fed wild $Y = 0.1344(x) + 0.201$; F1 diet-fed wild $Y = 0.1228(x) + 0.128$; F1 prey-fed lab $Y = 0.1325(x) + 0.204$; F1 diet-fed lab $Y = 0.090(x) + 0.129$; F11 diet-fed lab $Y = 0.119(x) + 0.010$. The slope values for the linear regressions indicated that the prey-fed insects gained weight faster than the diet-fed insects, wild insects gained weight faster than lab insects and the F11 diet-fed lab insects gained weight faster than the F1 diet-fed lab insects.

Effect of Artificial Diet on Nymphal Survivorship. Mortality occurred only in the second stadium of the prey-fed lab insects, second and third stadia of the F1 and F11 diet-fed lab insects and the second stadium of the diet-fed wild insects (Table 2). In the second stadium a significant difference was found between diet-fed F1 and F11 lab insects (Log-rank test; Chi-square = 4.60, df = 1, $P = 0.032$), whereas there were no significant differences between prey-fed and diet-fed F1 lab insects (Chi-square = 0.27, df = 1, $P = 0.6023$) and between prey-fed and diet-fed F11 insects (Chi-square = 2.99, df = 1, $P = 0.0835$). Also, there was no significant difference between prey-fed and diet-fed F1 wild insects (Chi-square = 1, df = 1, $P = 0.3173$).

Effect of Artificial Diet on Nymphal Developmental Time. No significant difference between male and female nymphal developmental time was observed during the 15 d observation period ($F = 1.71$, df = 1, 133, $P = 0.1936$). Therefore, the data for male and female developmental time were combined for these analyses. Both insect colony and food source affected the nymphal developmental time (Table 3). The nymphal developmental time was significantly shorter by 5.35 d for prey-fed insects than for diet-fed insects ($F = 285.74$, df = 1, 1585, $P = 0.0001$) and significantly shorter by 1.92 d for wild insects than for lab insects ($F = 38.29$, df = 1, 1585, $P = 0.0001$).

Developmental time to adult in F1 nymphs fed diet was extended by an average of five and 4 d for lab and wild colonies, respectively, compared with prey-fed insects (Fig. 2). The mean developmental time from second stadium to adult was significantly shorter for lab and wild prey-fed insects than for the lab and wild diet-fed insects ($F = 1988.88$, df = 1, 133, $P < 0.0001$;

Table 2. Stage specific and generational mortality of *P. maculiventris* fed larval prey or a zoophytophagous diet

Colony	Treatment	2nd Instar[a]	3rd Instar[a]	4th Instar[a]	5th Instar[a]	Generation mortality[b]
Lab	Prey-fed F1 ($n = 60$)	6.6	0.00	0.0	0.0	6.6
	Diet-fed F1 ($n = 100$)	8.0	2.00	0.0	0.0	10.0
	Diet-fed F11 ($n = 100$)	1.00	2.00	0.0	0.0	3.0
Wild	Prey-fed F1 ($n = 100$)	0.00	0.00	0.0	0.0	0.0
	Diet-fed F1 ($n = 100$)	1.00	0.00	0.0	0.0	1.0

[a] Stage specific mortality = $(d_x/l_x)*100\%$ (d_x = the number of individuals dying in stage x and 1_x = the number of individuals alive at beginning of stage x.

[b] Generational mortality = $\Sigma(d_x/l_0)*100\%$: d_x = the number of individuals dying in stage x and l_0 = the number of individuals alive at beginning of egg stage.

Table 3. Effect of insect colony and food source on developmental time[a]

Main effect	1st Instar	2nd Instar	3rd Instar	4th Instar
Colony (wild vs. lab)	$F = 38.59$ ($P < 0.0001$) (df = 1,403)	$F = 9.13$ ($P = 0.0027$) (df = 1,396)	$F = 26.37$ ($P < 0.0001$) (df = 1,392)	$F = 14.54$ ($P < 0.0002$) (df = 1,388)
Food source (prey vs. diet)	$F = 79.12$ ($P = <0.0001$) (df = 1,157)	$F = 68.71$ ($P < 0.0001$) (df = 1,157)	$F = 96.63$ ($P < 0.0001$) (df = 1,157)	$F = 173.84$ ($P < 0.0001$) (df = 1,157)

[a] GLM analysis.

$F = 326.89$, df = 1, 155, $P < 0.0001$, respectively). Significant differences in developmental time within stadia for F1 prey-fed compared with F1 diet-fed insects were found in the lab colony at second ($F = 237.77$, df = 1, 145, $P < 0.0001$), third ($F = 169.26$, df = 1, 128, $P < 0.0001$), fourth ($F = 222.60$, df = 1,136, $P < 0.0001$), and fifth ($F = 210.83$, df = 1, 133, $P < 0.0001$) stadia, and in the wild colony at second ($F = 75.69$, df = 1, 157, $P < 0.0001$), third ($F = 53.72$, df = 1,157, $P < 0.0001$), fourth ($F = 86.67$, df = 1, 157, $P < 0.0001$), and fifth stadia ($F = 171.34$, df = 1, 157, $P < 0.0001$). A significant decrease occurred in the developmental time of diet-fed insects from second stadium to adult of F11 lab insects when compared with F1 lab insects ($F = 153.45$, df = 1, 177, $P < 0.0001$), with an average decrease of 3 d. Significant differences were also found for the mean developmental times between F1 and F11 lab insects within the third ($F = 17.64$, df = 1, 182, $P < 0.0001$), fourth ($F = 19.98$, df = 1, 179, $P < 0.0001$), and fifth ($F = 149.57$, df = 1, 175, $P < 0.0001$) stadia, whereas the second stadium showed no significant difference between generations ($F = 1.537$, df = 1, 188, $P = 0.217$).

The cumulative mean developmental time from second instar to adult for lab F1 prey-fed females was significantly lower than for lab F1, F6, and F11 diet-fed females (Table 4). The cumulative developmental time did decline significantly between F1 and F6 diet-fed insects, but not between F6 and F11 diet-fed females. For wild insects, there was a significant and consecutive increase in the mean developmental time for F1, F6, and F11 diet-fed females when compared with that for F1 prey-fed females. There were no significant differences in the mean developmental times between prey-fed lab and wild females ($F = 1.14$, df = 1, 37, $P = 0.2916$), and between F6 and F11 diet-fed lab and wild females ($F = 3.71$, df = 1, 37, $P = 0.618$; $F = 0.95$, df = 1, 38, $P = 0.3363$, respectively). In contrast, the mean developmental time for diet-fed F1 lab females was significantly higher than for diet-fed F1 wild females ($F = 176.74$, df = 1, 38, $P = 0.0001$).

Effect of Artificial Diet on Adult Fertility Table Parameters and Female Reproductive Parameters. Adult mortality of mated females, measured only during the experimental mating period, was variable across all treatments (data not shown), with no significant treatment effects (Chi-square = 2.0252, df = 3, $P = 0.5671$). Developmental time to adult for females used to measure reproductive parameters adhered to the same pattern as reported in the previous section on nymphal developmental time (Table 4); i.e., the developmental time to adult for diet-fed insects was extended by an average of five and 4 d for lab and wild colonies, respectively (Table 4), as compared with prey-fed insects.

The adult weight for F1 prey-fed lab females was higher than for F1 diet-fed lab females, but did not differ significantly from that of F6 and F11 diet-fed lab females (Table 4). In contrast, the mean adult weight for F1 prey-fed wild females was significantly higher

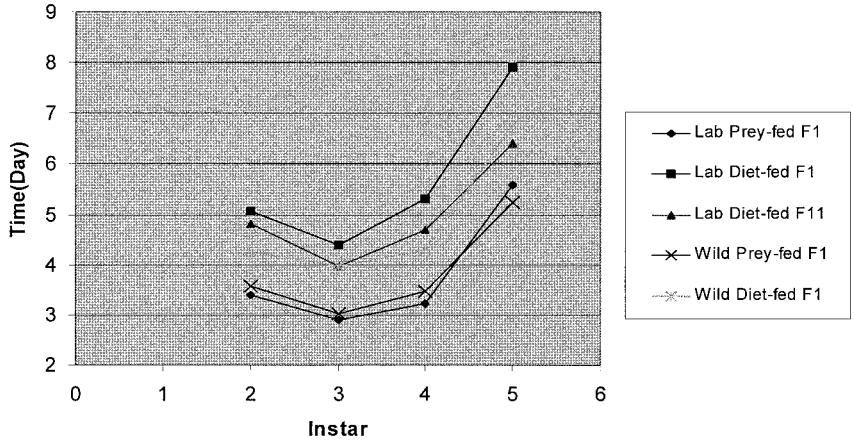

Fig. 2. Nymphal developmental time of F1 and F11 lab and F1 wild *P. maculiventris* reared on larval prey or a zoophytophagous diet. Data points represent means of each treatment.

Table 4. Average developmental time, adult weight, pre-oviposition period, eggs per female, and fertility of female *P. maculiventris* mated for fertility table experiment

Colony	Treatment	Cum. Dev. Time (day)[a]	Fem. weight (mg)[b]	Pre-Ovip (day)[c]	Eggs per Female[d]	Fertility[e]
Lab	Prey-fed F1 (n = 20)	14.89 ± 0.45c	93.30 ± 10.52a	6.00 ± 0.88a	230.0 ± 83.42a	1.00
	Diet-fed F1 (n = 20)	21.20 ± 0.41a	57.48 ± 8.88b	8.50 ± 1.27b	29.45 ± 15.11d	0.90
	Diet-fed F6 (n = 20)	20.26 ± 1.04b	88.15 ± 9.40a	6.95 ± 1.31a	114.8 ± 37.12c	1.00
	Diet-fed F11 (n = 20)	20.60 ± 1.18b	90.18 ± 5.38a	6.90 ± 0.96a	152.9 ± 28.02b	0.85
Wild	Prey-fed F1 (n = 20)	15.00 ± 0.00d	133.81 ± 9.34a	6.70 ± 0.65a	352.0 ± 47.23a	1.00
	Diet-fed F1 (n = 20)	18.95 ± 0.68c	87.1 ± 7.39d	8.40 ± 2.23b	107.3 ± 41.0b	1.00
	Diet-fed F6 (n = 20)	19.75 ± 0.63b	109.91 ± 6.2b	7.65 ± 1.18b	122.75 ± 46.8b	1.00
	Diet-fed F11 (n = 20)	20.40 ± 0.59a	91.81 ± 7.39c	7.50 ± 0.76b	116.3 ± 42.58b	1.00

Dev. Time, Female Weight, Pre-ovip, and Eggs per female values are reported as Lsmean ± SE developed from the GLM statistical analysis testing H = treatment, standard errors and probabilities calculated using a type III error (SAS Institute 1990). Means for each colony within same column followed by the same letter are not significantly different.

[a] Cumulative developmental time from second stadium to adult.

[b] Female weight measured 3 days after adult emergence.

[c] Number of days from emergence of adult to first oviposition.

[d] Average eggs per female (fertile females only) collected from day 5 to day 17 post-adult emergence.

[e] The proportion of females that laid fertile eggs (eggs that hatch) per number of total mated pairs.

than for F1, F6 and F11 diet-fed wild females. Also, the mean adult weights for F1, F6, and F11 wild females were significantly higher than the F1, F6 and F11 lab females, respectively, and the mean adult weight for F1 prey-fed wild females was significantly higher than for all other weights across both colonies.

The mean preovipositional period for F1 prey-fed lab females was significantly shorter than for F1 diet-fed lab females, but was not significantly different from F6 and F11 diet-fed females (Table 4). For wild insects, the mean preovipositional period for F1 prey-fed females was lower than for F1, F6, and F11 diet-fed females. There was no significant difference between the preovipositional period for F1 prey-fed lab and wild females (F = 0.23, df = 1, 37, P = 0.6349). However, the mean preovipositional period for F1 prey-fed lab females was significantly shorter than for F1, F6, and F11 diet-fed wild females (F = 23.95, df = 1, 38, P = 0.0001; F = 22.56, df = 1, 38, P = 0.0001; F = 54.71, df = 1, 38, P = 0.0001, respectively).

The mean number of eggs laid per female during the first 17 d after adult emergence for lab F1 prey-fed insects was significantly higher than for diet-fed lab females in each generation (Table 4). There was a significant and progressive increase in the mean number of eggs per female for lab F1, F6, and F11 diet-fed insects. For wild insects, the mean number of eggs per F1 prey-fed female was significantly higher than for diet-fed wild females in each generation tested. However, there was no significant difference in the mean number of eggs per female for wild F1, F6, and F11 diet-fed insects. The wild prey-fed F1 females laid significantly more eggs than the lab prey-fed females (F = 52.45, df = 1, 37, $P < 0.0001$). However, the lab F1 prey-fed females laid significantly more eggs than the wild F1, F6, and F11 diet-fed females (F = 24.82, df = 1, 37, $P < 0.0001$; F = 22.29, df = 1, 37, $P < 0.0001$; F = 23.10, df = 1, 37, $P < 0.0001$, respectively).

Rearing on the artificial diet lowered the fertility of the lab F1 and F11 females by 10 and 15%, respectively, when compared with F1 prey-fed insects (Table 4). However, there were no significant differences between these generations (Chi-square = 5.5949, df = 3, P = 0.1331). No decrease in fertility occurred in diet-fed wild females, compared with prey-fed insects.

The mean reproductive rates (R_0) were higher for the prey-fed insects than for the diet-fed insects, with the wild colony having the highest value, because the values of both the l_x and m_x were higher for the wild colony (Table 5). Over successive generations reared on the diet, the R_0 increased for lab insects, but re-

Table 5. Fertility table parameters for *P. maculiventris* maintained on larval prey or a zoophytophagous diet

Colony	Treatment	Egg (R_0[a])	Mean generation time (T[b]) (day)	Rate of Increase (r[c])
Lab	Prey-fed	59.48	33.30	1.0011
	Diet-fed (F1)	5.45	38.15	0.8929
	Diet-fed (F6)	23.30	41.15	1.004
	Diet-fed (F11)	34.90	40.05	1.0027
Wild	Prey-fed	178.83	34.69	1.1884
	Diet-fed (F1)	43.69	38.27	1.0001
	Diet-fed (F6)	46.85	38.90	1.0045
	Diet-fed (F11)	38.38	39.17	0.9979

[a] $R_0 = \Sigma l_x m_x$

l_x: the proportion of mated females alive at time x; m_x: the average daily number of eggs laid

[b] $T = (\Sigma \text{ age}^* l_x m_x)/R_0$

[c] r: Intrinsic increase Rate = $(\Sigma \exp(-r_R * \text{age}) * l_x m_x$; where $r_R = \ln(R_0)/T$.

Table 6. Doubling time and cost of rearing for *P. maculiventris* reared on larval prey or a zoophytophagous diet

Colony	Treatment	Doubling Time (T_d)[a] (day)	TCG[b] ($)	Cost/egg[c] ($)	Cost of doubling[d] ($)
Lab	Prey-fed	5.5451	$245.905	$0.02098	$40.943
	Diet-fed (F1)	14.5926	$277.980	$0.2662	$106.300
	Diet-fed (F6)	8.9785	$274.218	$0.0525	$59.344
	Diet-fed (F11)	7.7187	$274.290	$0.0371	$52.856
Wild	Prey-fed	4.636	$206.26	$0.0065	$27.40
	Diet-fed (F1)	6.91074	$221.09	$0.0253	$39.92
	Diet-fed (F6)	6.91764	$226.01	$0.0216	$40.18
	Diet-fed (F11)	7.32714	$230.01	$0.0265	$40.90

[a] T_d Doubling Time = $\ln(2)/(r_G)$.
[b] TCG = Cost of raw material required to rear one generation.
[c] Cost of Doubling = TCG*(T_d generation) where T_d generation = $\ln(2)/(r_G*T)$.
[d] Cost/egg = TCG/(e*f*nf*a*s).

mained relatively constant for the wild insects. In both lab and wild colonies, the mean generation time (T) was longer for diet-fed insects than for prey-fed insects, but remained relatively constant over successive generations reared on the diet. The intrinsic rate of increase (r) was higher for the prey-fed lab colony than for the F1 generation reared diet, but lower than for the diet-fed F6 and F11 generations. In contrast, r was higher in the wild colony for the prey-fed insects than for any generation of diet-fed insects. The values of R_0 in the wild diet-fed insects and of T and r in the lab and wild diet-fed insects are comparable to the respective values in the lab prey-fed insects.

Effect of Artificial Diet on Doubling Time and Cost of Rearing. Doubling time in generations (T_d) was shorter for prey-fed insects than for diet-fed insects, with the wild colony having the lowest values (Table 6). The doubling time decreased in lab insects, but remained relatively constant for the wild insects, over successive generations of rearing on the diet. In both lab and wild colonies, the cost of raw materials (total cost/generation) required to feed one generation was higher for diet-fed insects than for prey-fed insects, and remained relatively constant over successive generations of rearing on the diet. The higher number of eggs laid, shorter mean generation time and faster doubling time for prey-fed insects, resulted in lower values for the cost per egg and the cost to double population size (the realized cost of rearing) for diet-fed insects. The realized cost of rearing was lowest for the prey-fed wild colony (Table 6; Fig. 3). The intercepts of the linear and exponential extrapolations of the rearing costs of diet-fed lab insects over consecutive generations with the cost of rearing prey-fed lab insects indicate that the cost of rearing insects on the diet would approximate the cost of rearing insects on prey after ≈12 and 14 generations, respectively (Fig. 3).

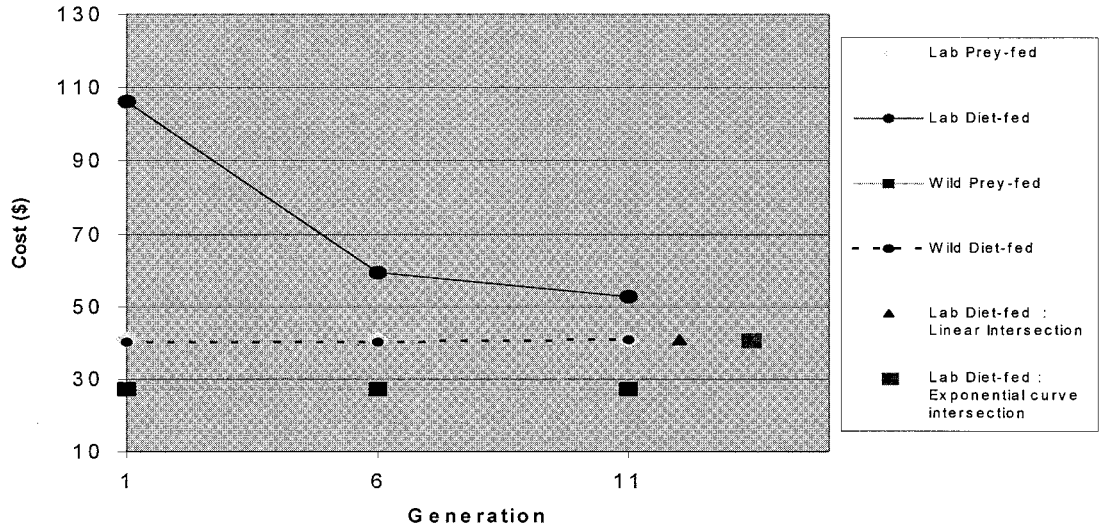

Fig. 3. Cost of doubling of F1, F6 and F11 lab and wild *P. maculiventris* reared on larval prey or a zoophytophagous diet. Equation for the estimated linear regression: cost = 104.89–5.344 × (generation). Equation for the estimated exponential curve:cost = 104.58 exp(−0.06987 × [generation]). The determinant coefficients (R^2) for the linear regression and exponential curve are 0.8385 and 0.8703, respectively.

Discussion

There were only a few instances of mortality of the prey-fed and diet-fed insects for both the lab and wild colonies. Mortality was limited to the second and third stadia in all treatments, and a significant difference was found only in the second stadium between the diet-fed F1 and F11 lab insects. Female mortality in both colonies was also not significantly affected by the artificial diet. Weight gain in diet-fed nymphs during both the F1 and F11 generations was slower than that in prey-fed nymphs for both lab and wild colonies. It is interesting to note that the weight gain in diet-fed lab insects increased in the F11 generation compared with the F1 generation, suggesting that adaptation or selection had occurred.

The developmental time to adult was prolonged for insects reared on the zoophytophagous diet, as was the case with other insect-free artificial diets tested for *P. maculiventris* (De Clercq and Degheele 1992, De Clercq et al. 1998a, Wittmeyer and Coudron 2001). The prolongation of development persisted from F1 to F11 generations in both lab and wild colonies. However, a significant decrease was observed in the developmental time of diet-fed lab insects between the F1 and F11 generations. This information indicates that sensitivity of an insect to an artificial diet, as measured by nymphal developmental time, may vary from one generation to another, and from one colony to another. Hence, developmental time alone may not be a reliable indicator of suitability of a diet.

To evaluate the true efficacy of the zoophytophagous diet for the continuous rearing of *P. maculiventris*, the realized cost of rearing was calculated (Thompson 1999, Wittmeyer and Coudron 2001). In this study the estimated cost of raw materials and labor to produce the artificial diet was low compared with the cost of raising the lepidopteran host (*T. ni*). However, by F12 to F14 that lower cost of materials and labor was sufficient to offset the projected added cost of prolonged development and reduced fecundity in the diet-fed lab insects, bringing the realized cost of rearing on this artificial diet in line with that of rearing on natural prey. In contrast, the lower developmental time and higher fecundity of the prey-fed wild insects lowered the realized cost of rearing these insects to ≈65% of the cost of rearing wild insects on the artificial diet over the 11 generations tested.

Both the reproductive rates and the mean generation times were negatively impacted by the artificial diet, leading to a lower intrinsic rate of increase and longer doubling time. This was most pronounced in the lab colony versus the wild colony. However, both of these values improved for the lab colony with subsequent generations of rearing on the diet. Consequently, the analysis of both the cost per egg and the cost of doubling based on life and fertility table parameters presented in this study revealed an initial increase in the cost to rear *P. maculiventris* on the artificial diet, with the highest cost associated with the early generations of lab insects. Overall, the performance of the wild colony on the artificial diet more closely approximated the performance of prey-fed insects, as compared with the lab colony, suggesting that better results may be obtained by mass rearing a wild colony. After rearing on the artificial diet for 11 generations, the cost of rearing the lab and wild insects on the diet approximated that of rearing lab insects on prey, and was ≈1.2 times higher than rearing wild insects on prey.

The cost of rearing of *P. maculiventris* on the zoophytophagous diet approached the cost of rearing on natural prey, and the performance was better than previously recorded when reared on the blended buffered mixture of beef liver and whole egg (Wittmeyer and Coudron 2001). When comparing the performance on the two artificial diets, the developmental time was shorter and the percentage egg hatch and survival of second instar nymphs was higher for the zoophytophagous diet. Additionally, both domesticated (lab) and field (wild) colonies (currently at F25 and F21, respectively) have shown an acceptance of and good development and sustained growth on the zoophytophagous diet. Therefore, the expectation is that the zoophytophagous diet will perform well when used to rear other colonies of *Podisus*, and that the success reported here is not an anomaly of a particular laboratory colony.

The zoophytophagous diet was developed for the rearing of *P. maculiventris.* Further improvements to the formulation and presentation are feasible and would likely enhance the cost-effective use of the diet for the mass rearing of *P. maculiventris.* Additionally, *Perillus bioculatus* (Fabricius), a predator of Colorado potato beetle, has been maintained on the zoophytophagous diet for 20 generations, showing a gradual improvement in life table parameters (T.A.C., unpublished data). Also, the following insects were found to feed, mate and produce viable eggs (i.e., develop from eggs oviposited by field-collected adults to egg-laying adults) when maintained solely on the zoophytophagous diet (TAC, unpublished data): the entomophagous insects, the lady beetle, *Coleomegilla maculata* De Geer, the big-eyed bug, *Geocoris punctipes* (Say), the common green lacewing, *Chrysoperla carnea* Stephens; and the phytophagous pentatomids, the green stink bug, *Acrosternum hilare* (Say), *Thyanta custator accerra* McAtee, and the one spotted stink bug, *Euschistus variolarius* (Palisot de Beauvois). Therefore, the zoophytophagous diet would appear to have potential for the artificial rearing of several insects, and should constitute a good starting formulation for pentatomids and other predators.

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References Cited

Abou-Setta, M. M., R. W. Sorrell, and C. C. Childers. 1986. Life 48: a basic computer program to calculate life table parameters for an insect or mite species. Florida Entomol. 69: 690–697.

Adams, T. S. 2000a. Effects of diet and mating status on ovarian development in a predaceous stink bug, *Perillus bioculatus* (Hemiptera: Pentatomidae). Ann. Entomol. Soc. Am. 93: 529–535.

Adams, T. S. 2000b. Effect of diet and mating on oviposition in the twospotted stinkbug, *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 93: 1288–1293.

Bellows, T. S. Jr., R. G. Van Driesche, and J. S. Elkinton. 1992. Life table construction and analysis in the evaluation of natural enemies. Annu. Rev. Entomol. 37: 587–614.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26.

Carpenter, J. E., and P. D. Greany. 1998. Comparative development and performance of artificially reared versus host-reared *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) wasps. Biol. Control 11: 203–208.

Cobben, R. H. 1978. Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. Meded. Landbouwhogesch. Wageningen. 78–5.

Conover, W. J., and R. L. Iman. 1981. Rank transformation as a bridge between parametric and nonparametric statistics. Am. Statistician 35: 124–128.

Debolt, J. W. 1982. Meridic diet for rearing successive generations of *Lygus hesperus.* Annu. Entomol. Soc. 75: 119–122.

De Clercq, P., and D. Degheele. 1992. A meat-based diet for rearing the predatory stinkbugs *Podisus maculiventris* and *Podisus sagitta* (Heteroptera: Pentatomidae). Entomophaga 37: 149–157.

De Clercq, P., and D. Degheele. 1997. Effects of mating status on body weight, oviposition, egg load, and predation in the predatory stink bug *Podisus maculiventris* (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 90: 121–127.

De Clercq, P., F. Merlevede, and L. Tirry. 1998a. Unnatural prey and artificial diets for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). Biol. Control 12: 137–142.

De Clercq, P., F. Merlevede, I. Mestdagh, K. Vandendurpel, J. Mohaghegh, and D. Degheele. 1998b. Predation on the tomato looper *Chrysodeixis chalcites* (Esper) (Lep., Noctuidae) by *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Het., Pentatomidae). J. Appl. Entomol. 122: 93–98.

Drummond, F. A., R. L. James, R. A. Casagrande, and H. Faubert. 1984. Development and survival of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), a predator of the Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 1283–1286.

Fouly, A. H., M. Abou-Setta, and C. Childers. 1995. Effects of diet on the biology and life tables of *Typhlodromalus peregrinus* (Acari: Phytoseiidae). Environ. Entomol. 23: 870–874.

Glenister, C. S. 1998. Predatory heteropterans in augmentative biological control: an industry perspective, pp. 199–208. *In* M. Coll and J. R. Ruberson [eds.], Predatory Heteroptera: Their Ecology and Use in Biological Control. Proceedings: Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD. 233p.

Glenister, C. S., and M. P. Hoffmann. 1998. Mass-reared natural enemies: Scientific, technological, and informational needs and considerations, pp. 242–247. *In* R. Ridgway, M. P. Hoffmann, M. N. Inscoe, and C. S. Glenister [eds.], Mass-Reared Natural Enemies: Application, Regulation, and Needs. Proceedings: Thomas Say Publications in Entomology. Entomol. Soc. Of Amer., Lanham, MD. 332p.

Hansen, D. L., H. F. Brodsgaard, and A. Enkegaard. 1999. Life table characteristics of *Macrolophus caliginosus* preying upon *Tetranychus urticae.* Entomol. Exp. Appl. 93: 269–275.

Hodek, I., and A. Honek. 1996. Ecology of Coccinellidae. Kluwer. Dordrecht, The Netherlands. 464p.

Hough-Goldstein, J. A. 1998. Use of predatory pentatomids in integrated management of the Colorado potato beetle, pp. 209–224. *In* M. Coll and J. R. Ruberson [eds.], Predatory Heteroptera: Their Ecology and Use in Biological Control. Proceedings: Thomas Say Publications in Entomology. Entomological Society of America, Lanham MD. 233p.

Hough-Goldstein, J., and D. McPherson. 1996. Comparison of *Perillus bioculatus* and *Podisus maculiventris* (Heteroptera: Pentatomidae) as potential control agents of the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 89: 1116–1123.

Landahl, J. T., and R. B. Root. 1969. Differences in the life tables of tropical and temperate milkweed bugs, genus *Oncopeltus* (Hemiptera: Lygaeidae). Ecology 50: 734–737.

McPherson, J. E. 1980. A list of the prey species of *Podisus maculiventris* (Hemiptera: Pentatomidae). Great Lakes Entomol. 13: 17–24.

McPherson, J. E. 1982. The Pentatomoidea (Hemiptera) of Northeastern North America with emphasis on the fauna of Illinois. Southern Illinois University Press, Carbondale, IL. 240p.

Miles, P. W. 1972. The saliva of Hemiptera. Adv. Insect Physiol. 9: 183–256.

Mukerji, M. K., and E. J. LeRoux. 1969. A quantitative study of food consumption and growth of *Podisus maculiventris* (Hemiptera: Pentatomidae). Can. Entomol. 101: 387–403.

Richard, D. R., and E. W. Evans. 1998. Reproductive response of aphidophagous lady beetles (Coleoptera: Coccinellidae) to non-aphid diets: an example from alfalfa. Ann. Entomol. Soc. 19: 632–640.

Rojas, M. G., J. A. Morales-Ramos, and E. G. King. 2000. Two meridic diets for *Perillus bioculatus* (Heteroptera: Pentatomidae), a predator of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Biol. Control 17: 92–99.

Ruberson, J. R., and M. Coll. 1998. Res. needs for the predaceous Heteroptera, pp. 225–233. *In:* M. Coll and J. R. Ruberson [eds.], Predatory Heteroptera: Their Ecology and Use in Biological Control. Proceedings Thomas Say Publications in Entomology. Entomological Society of America, Lanham MD. 233p.

Ruberson, J. R., Tauber, M. J., and Tauber, C. A. 1986. Plant feeding by *Podisus maculiventris* (Heteroptera: Pentatomidae): effect on survival, development, and preoviposition period. Environ. Entomol. 15: 894–897.

SAS Institute. 1989–1996. SAS (r) Proprietary Software. Release 6:12, Cary, NC.

SAS Institute. 1982. User's guide: Basic 1982 Edition, Cary, NC.

SAS Institute. 1995. Survival analysis using the SAS system. A Practical Guide. Cary, NC.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591–610.

Souissi, R., and B. Le Ru. 1997. Comparative life table statistics of *Apoanagyrus lopezi* reared on the cassava mealybug *Phenacoccus manihoti* fed on four host plants. Entomol. Exp. Appl. 85: 113–119.

Thompson, S. N. 1999. Nutrition and culture of entomophagous insects. Annu. Rev. Entomol. 44: 561–592.

Valicente, F. H., and R. J. O'Neil. 1995. Effects of host plants and feeding regimes on selected life history characteristics of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Biol. Control 5: 449–461.

Waddill, V., and M. Shepard. 1975. A comparison of predation by the pentatomids, *Podisus maculiventris* (Say) and *Stiretrus anchorago* (F.), on the Mexican bean beetle, *Epilachna varivestis* Mulsant. Ann. Entomol. Soc. Am. 68: 1023–1027.

Wittmeyer, J. L., T. A. Coudron, and T. Adams. 2001. Ovarian development, fertility and fecundity in *Podisus maculiventris* Say (Heteroptera: Pentatomidae): an analysis of the impact of nymphal, adult, male and female nutritional source on reproduction. Invertebr. Repro. Dev. 39: 9–20.

Wittmeyer, J. L., and T. A. Coudron. 2001. Life table parameters, reproduction rate, intrinsic rate of increase, and estimated cost of rearing *Podisus maculiventris* (Heteroptera: Pentatomidae) on an artificial diet. J. Econ. Entomol. 94: 1334–1352.

Yeargan, K. V. 1998. Predatory Heteroptera in North America Agroecosystems: An overview, pp. 7–20. *In* M. Coll and J. R. Ruberson [eds.], Predatory Heteroptera: Their Ecology and Use in Biological Control. Proceedings: Thomas Say Publications in Entomology. Entomological Society of America, Lanham MD. 233p.

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